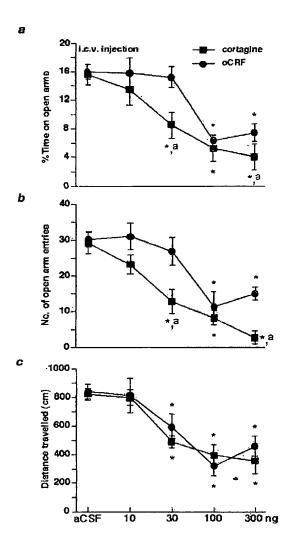
Docket No.: 0147-0277PUS1

App No.: NEW Inventor: Joachim SPIESS et al.

Title: NOVEL CORTICOTROPIN-RELEASING FACTOR

RECEPTOR 1 (CRFR1) AGONIST NEW SHEET Sheet 1 of 6

Figure 1/6



App No.: NEW Inventor: Joachim SPIESS et al. Docket No.: 0147-0277PUS1

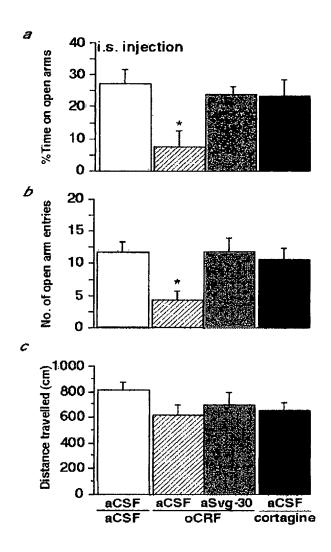
Title: NOVEL CORTICOTROPIN-RELEASING FACTOR

RECEPTOR 1 (CRFR1) AGONIST

NEW SHEET

Sheet 2 of 6

Figure 2/6



App No.: NEW Docket No.: 0147-0277PUS1

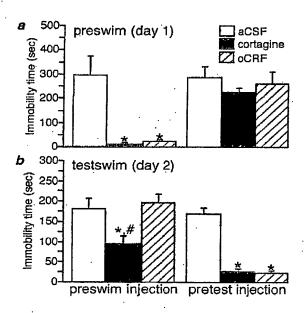
Inventor: Joachim SPIESS et al.

Title: NOVEL CORTICOTROPIN-RELEASING FACTOR
RECEPTOR 1 (CRFR1) AGONIST

NEW SHEET

Figure 3/6

Sheet 3 of 6



Docket No.: 0147-0277PUS1

App No.: NEW Inventor: Joachim SPIESS et al.

Title: NOVEL CORTICOTROPIN-RELEASING FACTOR
RECEPTOR 1 (CRFR1) AGONIST
NEW SHEET
She

Sheet 4 of 6 Figure 4/6

Table 1. Sequence alignment of oCRF, h/rCRF, Svg and their chimeric analogs

Sequence	SOEPPISLUL TEHEBREVEE MUNABOLAGO AHSNRKLLDI A	SEEPPISLUL TEHILIRENTE MARABOURGO AHSWRKLMEI I	ZGPPISIDL SLEDINKATE LEKOEKOKOO AANNRLLLDT I	SEEPPISLDL TFHILKKNITE TEKOEKEKOO AHSNRKLMEI I	SEEPPISLUL TFHUNKEVIE MARAEQUAGO AANNRLLLDT I	ZGPPISIDL SLEINKHYLE MARKEGINGG AANWRLLLDT I	ZGPPISIDL SLEIGRKWIP LEKODKEKOO AHSNRKLMEI I	ZGPPISIDL SLEINKEVLE NAKARGIAGO AANNKLLLDT A	ZGPPISIDL SLEUDENGENGENEGINGO AANNRLLLDT A
S	SQEPPISLDL TFHELFEVE	SEEPPISLDL TFHURKEY	ZGPPISIDL SLEDGREG	SEEPPISLDL TFHEFFR	SEEPPISLDL TFHDOREN	ZGPPISIDL SLEDKRY	ZGPPISIDL SLETCHEN	ZGPPISIDL SLEDIKEN	ZGPPISIDL SLEGDEEN
No. Peptide	oCRF ¹⁴¹	h/rCRF ¹⁻⁴¹	Svg ¹⁻⁴⁰	[h/rCRF ^{1·13}]x[Svg ¹³⁻²⁹]x[h/rCRF ³¹⁻⁴ 1]	[h/rCRF ¹⁻³⁰]x[Svg ³⁰⁻⁴⁰]	[Svg ¹⁻¹²]x[h/rCRF ¹⁴⁻³⁹]x[Svg ³⁰⁻⁴⁰]	[Svg ¹⁻²⁹]x[h/rCRF ³¹⁻⁴¹]	[Ala ⁴⁰][Svg ⁺⁻¹²]x[h/rCRF ¹⁴⁻³⁰]x[Svg ³⁰⁻³⁹]	[Glu ²¹ , Ala ⁴⁰][Svg ¹⁻¹²]x[h/rCRF ¹⁴⁻³⁰]x[Svg ³⁰⁻³⁹]

The three main building blocks of the chimeric peptides, the N-terminal, central and C-terminal domains, are indicated. Sequences derived from h/rCRF, Svg, and oCRF are underlayed in grey, black and white, respectively. Z, pyroglutamic acid. Compound 9 is cortagine.

App No.: NEW

Docket No.: 0147-0277PUS1

nventor: Joachim SPIESS et al.

Fittle: NOVEL CORTICOTROPIN-RELEASING FACTOR

RECEPTOR 1 (CRFR1) AGONIST

NEW SHEET

Sheet 5 of 6

Figure 5/6

0.54 (0.38-0.71) 450 (420-480) 1.9 (1.8-2.0) Table 2. Binding affinites of oCRF, hrrCRF, Svg and their chimeric analogs 57 (45-70) CRFBP > 1000 9 9 2 9 0.69 (0.45-0.93) 0.98 (0.59-1.4) 700 (490-910) 400 (360-450) 160 (120-200) 330 (140-530) 540 (480-590) 0.9 (0.72-1.1) mCRFR28 42 (25-59) 0.52 (0.29-0.74) 0.47 (0.18-0.77) 1.8 (0.75-2.8) 2.0 (0.80-3.1) .8 (1.1-2.4) .6 (1.3-1.9) 1.8 (1.4-2.1) 2.6 (1.6-3.4) 9.5 (4.8-14) ICso, nM CRFR1 Compound ₽ ₩

ICso values are the mean of at least four experiments performed in duplicate. 95 % confidence intervals are given in parentheses. Compound 2-7 were dissolved in 10 mM aqueous acetic acid, whereas compound 1, 8, and 9 were dissolved in phosphate buffered saline (see Results section for details). ND, not determined.

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The intermediate compounds of the agonist development were not tested for their affinity to rCRFBP

Binding data taken from Eckart et al., 2001 Compound 9 is cortagine.

Docket No.: 0147-0277PUS1

Inventor: Joachim SPIESS et al.

Title: NOVEL CORTICOTROPIN-RELEASING FACTOR
RECEPTOR 1 (CRFR1) AGONIST
NEW SHEET
She

Sheet 6 of 6

∂igure 6/6

Table 3. Comparison of the pharmacological and physicochemical properties of cortagine and oCRF

p/-value 4.8 6.4 С_{тах}, µМ > 1000 > 1000 8.8 (6.0-12) mCRFR2B Biological potency ECso, nIM 16 (11-20) 0.47 (0.14-0.80) 0.18 (0.10-0.26) rCRFR1 cortagine Peptide OCRF

Cso and ECsovalues are the mean of at least four experiments performed in duplicate. 95 % confidence intervals are given in parentheses. Isoelectric points were determined by IEF.

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